PRACTICAL MANUAL OF BIOCHEMISTRY

First Edition

Dr. G. Durai Muthu Mani

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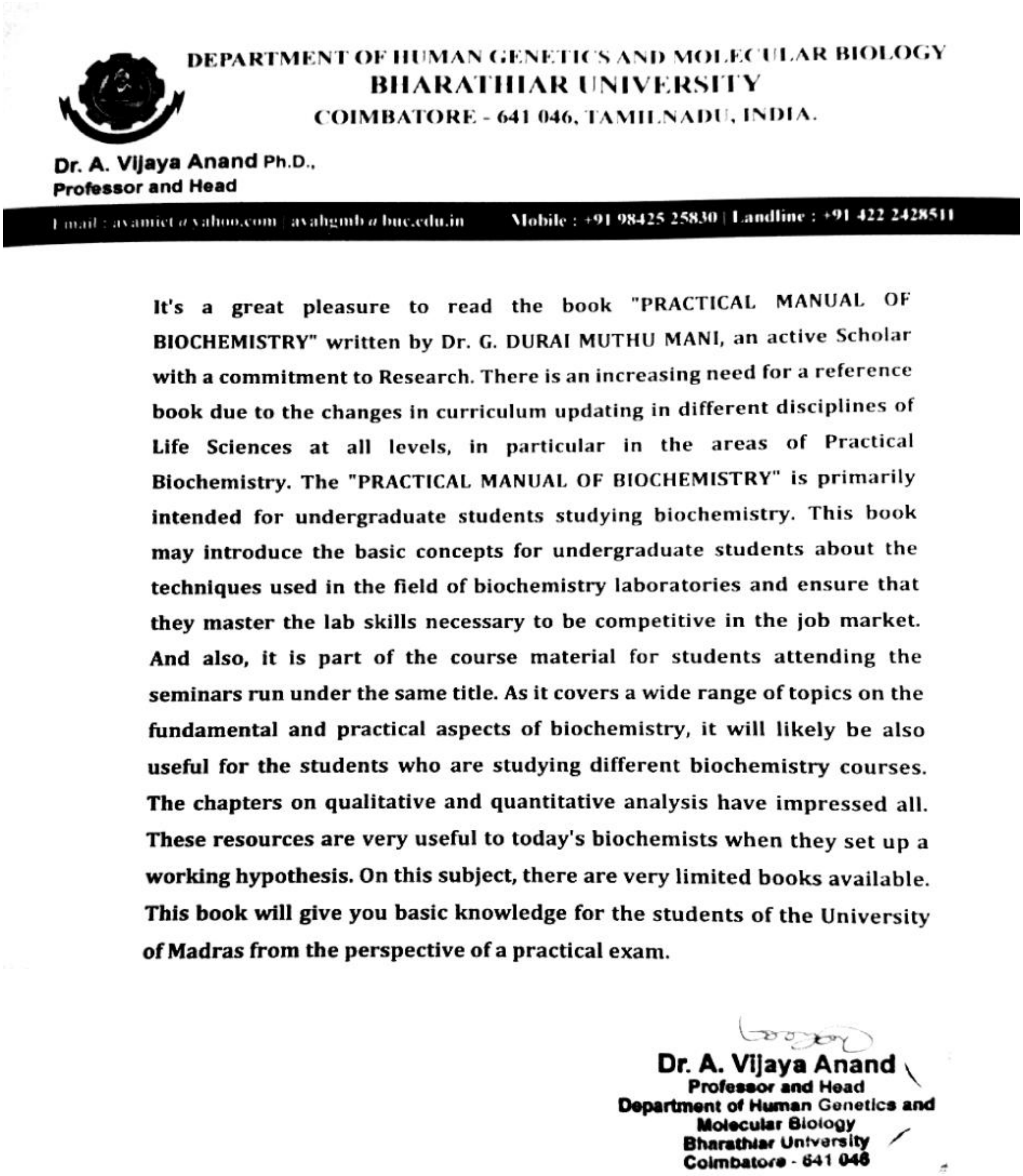
Foreword



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It's a great pleasure to read the book "PRACTICAL MANUAL OF BIOCHEMISTRY" written by Dr. G. DURAI MUTHU MANI, an active Scholar with a commitment to Research. There is an increasing need for a reference book due to the changes in curriculum updating in different disciplines of Life Sciences at all levels, in particular in the areas of Practical Biochemistry. The "PRACTICAL MANUAL OF BIOCHEMISTRY" is primarily intended for undergraduate students studying biochemistry. This book may introduce the basic concepts for undergraduate students about the techniques used in the field of biochemistry laboratories and ensure that they master the lab skills necessary to be competitive in the job market. And also, it is part of the course material for students attending the seminars run under the same title. As it covers a wide range of topics on the fundamental and practical aspects of biochemistry, it will likely be also useful for the students who are studying different biochemistry courses. The chapters on qualitative and quantitative analysis have impressed all. These resources are very useful to today's biochemists when they set up a working hypothesis. On this subject, there are very limited books available. This book will give you basic knowledge for the students of the University of Madras from the perspective of a practical exam.

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Department of Human Genetics and
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Preface

The present book entitled "Practical Manual of Biochemistry" has been written with the feeling that it will usefully serve the purpose of B.Sc. and M.Sc students. Basic as well as modern views are considered. Informative & understandable diagrams have been incorporated in this book.

The content is very simple. In preparation of book many books & papers have been consulted. I hope that this book will continue to serve its purpose in understanding the basic principles of Biochemistry & securing at the same time maximum marks in examination by the students.

I am also grateful to my publishers.

Criticism & suggestions for further improvement of this book are most cordially invited.

About author

Dr. G. Durai Muthu Mani is Assistant Professor in Biochemistry working at SRM College of Arts and Sciences, Kattankulathur, Chengalpet District, Tamil Nadu, India affiliated to University of Madras, Chennai. He has completed his under graduation in Bio Chemistry in S. Chattanatha Karayalar College of Arts and Science, Tenkasi. He did his Master degree in Biochemistry at Biochemistry in MIET Arts & Science College, Trichy and. M.Phil Biochemistry Bharathidasan University, Trichy. He completed his Ph.D. degree from Biochemistry in Manonmaniam Sundaranar University, Tirunelveli. He has 15 years working experience as a Assistant Professor. He had published about 3 research papers in national & international journals, also attended seminar, national and international conferences at the various institutes.

SUBJECT CODE: TAP4A

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DISTRIBUTION OF MARKS

- Practical – 100 marks
- Internals - 40 marks
- Externals -60 marks

1. **Externals - 60**

a) Record- 10 marks.
b) Qualitative – 25 (Compulsory) marks.
c) Titration/ Colorimetry- 25 marks.

2. **Internship:**

Students should be sent for Internship training (During summer Holidays) for 15 days and co-ordinated by staff members of the Department. Internship report to be evaluated by HOD and senior members of the department and 20 marks will be allotted for internship and 20 marks for internals.

BASIC CONCEPTS

BASIC RULES

a) Always wear lab coat while you enter the laboratory.

b) Do not measure solutions of bases & acids with mouth pipette.

c) Do not pour water to acid (especially H_2SO_4) when you prepare acid solution.

d) Do not eat or drink in the lab.

e) Do not keep food in the lab refrigerator.

f) Use test tubes holder while handling acids.

g) Cuvette wipe the lower half of the tube or cuvette free from moisture, lint and finger marks before placing it in the colorimeter instrument.

Important points

1. Normality (N):

The normality is the number of gram equivalent of the solute in 1 liter of the solution.

2. Molality (m)

The molal concentration (m) is the number of moles of the solute in 1 kg of the solvent.

3. Molarity (M)

The molar concentration gives the number of moles of the solute in 1 liter of the solution.

4. Calculation & conversion

1 g = 1000 mg
1 mg = 1000 µg

Conversion of

$\text{g} \longrightarrow$ mg multiply by 1000
$\text{mg} \longrightarrow$ g divide by 1000

Deciliter , dl = 100 ml

5. Qualitative analysis:

i) Qualitative - Qualitative analysis involves collecting & analyzing non - numerical data or to check the presence or absence of a substance.

ii)Quantitative- it is to analyze the actual moment of a substance.

iii) Carbohydrates - carbohydrates may be defined as poly hydroxy aldehydes or ketones or substances that yield one of these compounds on hydrolysis.

A) monosaccharides - Glucose , fructose , galactose & mannose.

Aldose sugar contains aldehyde group $\mathrm{R{-}CH{=}O}$

Ketose sugar contains keto group $\mathrm{RR'{-}C{=}O}$

B) disaccharides - sucrose , lactose & maltose.

C) polysaccharides - starch , glycogen.

iv) Reducing sugar:

They contain free aldehyde or ketone groups that are capable of reducing an oxidizing agent.

Eg: Maltose composed of two molecules of glucose.

v) Non reducing sugar:

They lack free aldehyde or ketone groups.

Eg: sucrose composed of one molecule of α-glucose and one molecule of β-fructose linked together by β-1,2-glycosidic linkage.

6. Amino acids:

Amino acids are simplest unit of protein molecules and they form the building blocks of protein.

Classification based on ‘R’ groups:

i). Aromatic amino acids:
  - Tyrosine
  - Tryptophan
  - Phenyl amine

ii). Sulphur containing amino acids:
  - Methionine
  - Cysteine
  - Cystine

iii). Hydroxyl group amino acid
  - Serine
  - threonine

iv).guanidino group - arginine

v).imidazole - histidine.

7. Colorimeter:

The working principle of the colorimeter is based on Beer-Lambert’s law which states that the amount of light absorbed is directly proportional to the concentration of the solution and the length of a light path through the solution.

8. Centrifugation: (Based on the principle of sedimentation)

It is a mechanical process which involves the use of the centrifugal force to separate particles from a solution according to their size, shape, density, medium viscosity and rotor speed.

9. Buffer:

A buffer is solution that can resists p^H change upon the addition of an acid or basic components.

p^H below 7 is acid
p^H above 7 is base
p^H 7 is considered as neutral.

10. Saponification number:

i. It is defined as the number of mg of KOH required to hydrolyse (saponify) 1g of fat or oil.
ii. It is used to measure average molecular size of the fatty acid present in fat or oil.

11. Acid number:

i. It is defined as the number of mg oh KOH required to completely neutralize free fatty acids present in 1g of fat or oil.
ii. Used to identify decomposition or bacterial contamination of fat or oil.

12. Iodine number:

Iodine number is defined as the number of grams of iodine absorbed by 100g of fat or oil.

Used to know the content of unsaturated fatty acid present in fat or oil.

13. Double distilled water:

Distilled water contains magnesium , phosphorus which may affect the estimation of certain compounds hence double distilled water is used in the estimation of phosphorus.

14.Chromatography:

Chromatography is a laboratory technique for the separation of a mixture.
Mixture dissolved in a fluid called mobile phase.
The phase over which the mobile phase passes is stationary phase.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance travelled by the solvent}}$$

Standard values:

S.NO	STANDARDS	R_f
SUGARS		
1.	Glucose	0.44
2.	Fructose	0.51
3.	Sucrose	0.42
4.	Lactose	0.17
AMINO ACIDS		
1.	Arginine	0.20
2.	Proline	0.43
3.	Tyrosine	0.45

15. Scope of practical biochemistry:

I) The findings of biochemistry are applied primarily in medicine, nutrition and agriculture.

II) In medicine, biochemists investigate the causes and cures of diseases.

III) Nutrition studies how to maintain health and rectify the nutritional deficiencies.

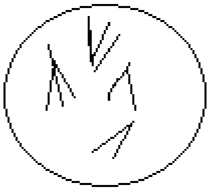
IV) In research field to develop drugs.

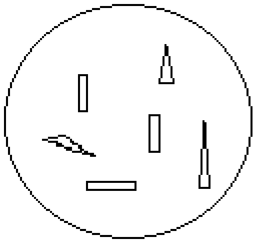
QUALITATIVE ANALYSIS

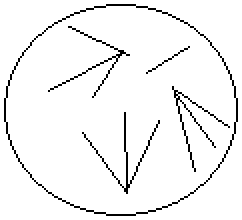
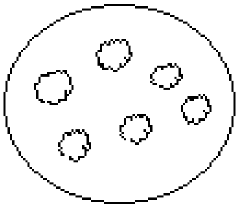
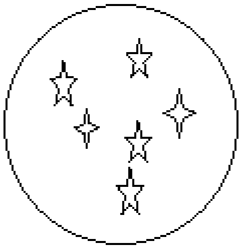
QUALITATIVE ANALYSIS
OF
CARBOHYDRATES

EX NO : 1 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (GENERAL PROCEDURE)

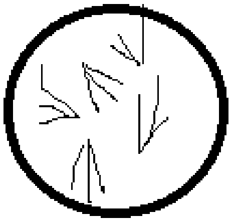
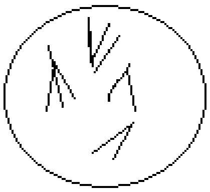
S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of ɑ-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	No red colour precipitate was formed.	Absence of monosaccharides.

5.	**Test for pentose sugar:** **Bial’s test-** To 1 ml of sugar solution , add 2ml of Bial’s reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar
6.	**Test for reducing sugar:** **A) Benedict’s test-** To 1ml of sugar solution , 2 ml of benedict’s reagent was added and kept in boiling water bath.	No brick red precipitate was formed.	Absence of reducing sugar
	B) Fehling’s test- To 1ml of sugar solution,add 1ml of Fehling’s reagent and kept in boiling water bath.	No red precipitate was formed.	Absence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff’s test-** To 1ml of sugar solution, add 2ml of Seliwanoff’s reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped glucosazone crystals were formed within 10-12 minutes. 	Presence of Glucose was confirmed.

		Yellow needle shaped fructosazone were formed within 5 minutes. Broken glass shaped galactosazone were formed within 25-30 minutes.  Yellow needle shaped mannosazone wee formed within 40-45 minutes. 	Presence of Fructose was confirmed. Presence of Galactose was confirmed. Presence of Mannose was confirmed.

		Yellow long needle shaped xylosazone crystals were formed within 30 minutes. 	Presence of Xylose was confirmed.
		Powder puff or cotton ball shaped lactosazone were formed within 45 minutes when the solution comes down to the room temperature. 	Presence of Lactose was confirmed.
		Sunflower shaped maltosazone was formed within 30 minutes ,when the solution comes down to room temperature. 	Presence of Maltose was confirmed.

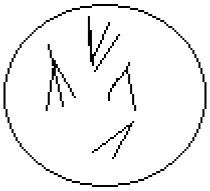
9.	**HYDROLYSIS OF SUGAR:** 5ml of sugar solution was treated with 1ml of 1N dilute hydrochloric acid. This was kept in boiling water bath for 15 minutes. A part of this solution was treated with iodine and heated for bluish violet colour, another part of the solution is treated with Barfoed's,Bial's,Fehling's &Seliwanoff's reagent. And the remaining test solution which was completely hydrolyzed was taken for osazone test.

S.NO	**EXPERIMENT**	**OBSERVATION**	**INFERENCE**
10.	**Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped glucosazone and fructosazone crystals were formed in 5minutes. 	Presence of sucrose was confirmed.
		Yellow needle shaped glucosazone crystals were formed within 5 minutes. 	Presence of starch was confirmed.

RESULT: The given sugar solution was systematically found.

EX NO : 2 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (GLUCOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution, 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution, add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped glucosazone crystals were formed within 10-12 minutes. 	Presence of Glucose was confirmed.

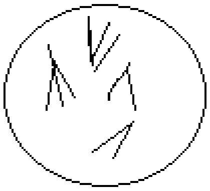
RESULT:

The given known sugar solution was systematically analyzed and was found to be

a) Monosaccharide.
b) Reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Glucose**.

EX NO : 3 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (FRUCTOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	Cherry red colour was formed.	Presence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped fructosazone were formed within 5 minutes. 	Presence of Fructose was confirmed.

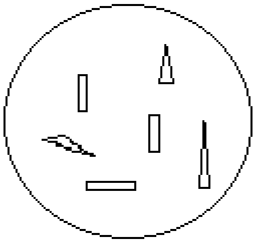
RESULT:

The given known sugar solution was systematically analyzed and was found to be

a) Monosaccharide.
b) Reducing sugar, ketose group.
c) Hexose sugar and confirmed as **Fructose**.

EX NO : 4 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (GALACTOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Broken glass shaped galactosazone were formed within 25-30 minutes. 	Presence of galactose was confirmed.

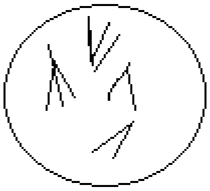
RESULT:

The given known sugar solution was systematically analyzed and was found to be

a) Monosaccharide.
b) Reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Galactose**.

EX NO : 5 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (MANNOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped mannosazone wee formed within 40-45 minutes. 	Presence of mannose was confirmed.

RESULT:

The given known sugar solution was systematically analyzed and was found to be

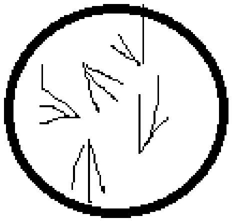
a) Monosaccharide.
b) Reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Mannose**.

EX NO : 6 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (SUCROSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	No red colour precipitate was formed.	Absence of monosaccharides.

5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar
6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath.	No brick red precipitate was formed.	Absence of reducing sugar
	B) Fehling's test- To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	No red precipitate was formed.	Absence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**HYDROLYSIS OF SUGAR:** 5ml of sugar solution was treated with 1ml of 1N dilute hydrochloric acid. This was kept in boiling water bath for 15 minutes. A part of this solution was treated with Barfoed's,Bial's,Fehling's &Seliwanoff's reagent and the remaining test solution which was completely hydrolyzed was taken for osazone test.		

9.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
10.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar
11.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath.	Brick red precipitate was formed.	Presence of reducing sugar
	B) Fehling's test- To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Red precipitate was formed.	Presence of reducing sugar
12.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	Cherry red colour was formed.	Presence of ketose sugar

13.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped glucosazone and fructosazone crystals were formed in 5minutes. 	Presence of sucrose was confirmed.

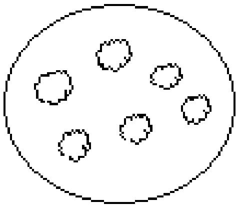
Result:

The given known sugar solution was systematically analyzed and was found to be

a) Disaccharide
b) Non-reducing sugar, aldose & ketose group.
c) Hexose sugar and confirmed as **Sucrose**.

EX NO : 7 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (LACTOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	No red colour precipitate was formed.	Absence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Powder puff or cotton ball shaped lactosazone were formed within 45 minutes when the solution comes down to the room temperature. 	Presence of lactose was confirmed.

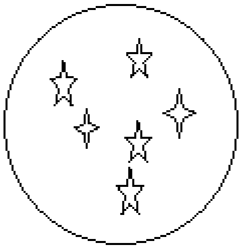
RESULT:

The given known sugar solution was systematically analyzed and was found to be

a) Disaccharide.
b) Reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Lactose**.

EX NO : 8 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (MALTOSE)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	Soluble in cold water.	Presence of monosaccharide and disaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	No blue colour was formed.	Absence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	No red colour precipitate was formed.	Absence of monosaccharides.
5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar

6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Brick red precipitate was formed. Red precipitate was formed.	Presence of reducing sugar Presence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Sunflower shaped maltosazone was formed within 30 minutes ,when the solution comes down to room temperature. 	Presence of maltose was confirmed.

RESULT:

The given known sugar solution was systematically analyzed and was found to be

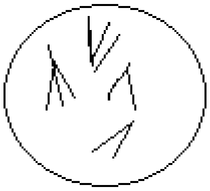
a) Disaccharide.
b) Reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Maltose**.

EX NO : 9 DATE :	QUALITATIVE ANALYSIS OF CARBOHYDRATES (STARCH)

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Solubility test:** The given solution was mixed with cold water.	In soluble in cold water.	Presence of polysaccharide.
2.	**Test for carbohydrates:** **Molisch's test-** To 2 ml of sugar solution , add 2-3 drops of α-naphthol solution and 1ml of concentrated and sulphuric acid along the sides of the test tube.	Violet colour ring was formed at the junction of two liquids.	Presence of carbohydrate was confirmed.
3.	**Test for polysaccharides:** **Iodine test-** To 1 ml of sugar solution , add 4-5 drops of iodine solution and the contents were mixed gently.	Blue colour was formed.	Presence of polysaccharides.
4.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	No red colour precipitate was formed.	Absence of monosaccharides.

5.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar
6.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath. **B) Fehling's test-** To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	No brick red precipitate was formed. No red precipitate was formed.	Absence of reducing sugar Absence of reducing sugar
7.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar
8.	**HYDROLYSIS OF SUGAR:** 5ml of sugar solution was treated with 1ml of 1N dilute hydrochloric acid. This was kept in boiling water bath for 15 minutes. A part of this solution was treated with Barfoed's,Bial's,Fehling's &Seliwanoff's reagent and the remaining test solution which was completely hydrolyzed was taken for osazone test.		

9.	**Test for monosaccharides :** **Barfoed's test-** To 1 ml of sugar solution, add 2 ml of Barfoed's reagent and kept in boiling water bath for 2-5 minutes.	Red colour precipitate was formed.	Presence of monosaccharides.
10.	**Test for pentose sugar:** **Bial's test-** To 1 ml of sugar solution , add 2ml of Bial's reagent and kept in boiling water bath.	No blue or emerald green colour complex was formed.	Absence of pentose sugar
11.	**Test for reducing sugar:** **A) Benedict's test-** To 1ml of sugar solution , 2 ml of benedict's reagent was added and kept in boiling water bath.	Brick red precipitate was formed.	Presence of reducing sugar
	B) Fehling's test- To 1ml of sugar solution,add 1ml of Fehling's reagent and kept in boiling water bath.	Red precipitate was formed.	Presence of reducing sugar
12.	**Test for ketose sugar:** **Seliwanoff's test-** To 1ml of sugar solution, add 2ml of Seliwanoff's reagent and kept in boiling water bath.	No cherry red colour was formed.	Absence of ketose sugar

13.	**Confirmatory test for carbohydrates** **Osazone test:** To 1ml of hydrolyzed sugar solution, add 0.5ml of phenyl hydrazine and kept in boiling water bath. The time taken for crystal formation was noted and a small amount of the precipitate was transferred to the clean microscopic slide. Excess water was blotted using filter paper. The slide was viewed under the microscope and the crystal structure was analyzed.	Yellow needle shaped glucosazone crystals were formed within 5 minutes. 	Presence of starch was confirmed.

Result:

The given known sugar solution was systematically analyzed and was found to be

a) Polysaccharide
b) Non-reducing sugar, aldose group.
c) Hexose sugar and confirmed as **Starch**.

QUALITATIVE ANALYSIS
OF
AMINO ACIDS

EX NO: 1 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (GENERAL PROCEDURE)

S.NO	**EXPERIMENTS**	**OBSERVATION**	**INFERENCE**
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	a) Purple or violet colour was formed. b) Yellow colour was formed.	Shows the presence amino acids with α-amino group. Shows the presence of amino acids like proline and hydroxy proline.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	Orange red colour was formed.	Shows the presence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** i. **Hopkin’s-cole’s test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	Violet colour ring was formed at the interphase.	Shows the presence of tryptophan
	ii. **Ehrlisch’s test:** To 1ml of test solution, 1mlof Ehrlisch’s reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	Red colour was formed	Shows the presence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	Violet colour ring was formed	Shows the presence of tryptophan.
5.	**Test for tyrosine:** i. **Millon’s test:** To 1 ml of test solution, 2-5 drops of millon’s reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	Red colour was formed	Shows the presence of tyrosine.

	ii. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	Green colour was formed.	Shows the presence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	Red colour was formed	Shows the presence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	Red colour was formed.	Shows the presence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **i. Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	Black precipitate was formed.	Shows the presence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	ii. Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	Red colour was formed.	Shows the presence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	Bottle green colour was formed.	Shows the presence of methionine.

Result:

The given sample was systematically analyzed and found to be **Amino Acids.**

EX NO: 2 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (TYROSINE)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	Orange red colour was formed.	Shows the presence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin's-cole's test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	No violet colour ring was formed at the interphase.	Shows the absence of tryptophan
	ii. **Ehrlisch's test:** To 1ml of test solution, 1mlof Ehrlisch's reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	No red colour was formed	Shows the absence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	No violet colour ring was formed	Shows the absence of tryptophan.
5.	**Test for tyrosine:** **a. Millon's test:** To 1 ml of test solution, 2-5 drops of millon's reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	Red colour was formed	Shows the presence of tyrosine.

	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	Green colour was formed.	Shows the presence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	Red colour was formed.	Shows the presence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	No red colour was formed.	Shows the absence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	No black precipitate was formed.	Shows the absence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	No red colour was formed.	Shows the absence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	No bottle green colour was formed.	Shows the absence of methionine.

Result:

The given sample was systematically analyzed and found to be **Tyrosine**

EX NO: 3 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (TRYPTOPHAN)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	Orange red colour was formed.	Shows the presence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin’s-cole’s test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	Violet colour ring was formed at the interphase.	Shows the presence of tryptophan
	ii. **Ehrlisch’s test:** To 1ml of test solution, 1mlof Ehrlisch’s reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	Red colour was formed	Shows the presence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	Violet colour ring was formed	Shows the presence of tryptophan.
5.	**Test for tyrosine:** **a. Millon’s test:** To 1 ml of test solution, 2-5 drops of millon’s reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	No red colour was formed	Shows the absence of tyrosine.

	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	No green colour was formed.	Shows the absence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	No red colour was formed	Shows the absence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	No red colour was formed.	Shows the absence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	No black precipitate was formed.	Shows the absence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	No red colour was formed.	Shows the absence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	No bottle green colour was formed.	Shows the absence of methionine.

Result:

The given sample was systematically analyzed and found to be **Tryptophan**

EX NO: 4 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (HISTIDINE)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	No orange red colour was formed.	Shows the absence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin's-cole's test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	No violet colour ring was formed at the interphase.	Shows the absence of tryptophan
	ii. **Ehrlisch's test:** To 1ml of test solution, 1mlof Ehrlisch's reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	No red colour was formed	Shows the absence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	No violet colour ring was formed	Shows the absence of tryptophan.
5.	**Test for tyrosine:** **a. Millon's test:** To 1 ml of test solution, 2-5 drops of millon's reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	No red colour was formed	Shows the absence of tyrosine.

	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	No green colour was formed.	Shows the absence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	Red colour was formed	Shows the presence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	No red colour was formed.	Shows the absence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	No black precipitate was formed.	Shows the absence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	No red colour was formed.	Shows the absence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	No bottle green colour was formed.	Shows the absence of methionine.

Result:

The given sample was systematically analyzed and found to be **Histidine**.

EX NO: 5 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (CYSTEINE)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	No orange red colour was formed.	Shows the absence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin’s-cole’s test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	No violet colour ring was formed at the interphase.	Shows the absence of tryptophan
	ii. **Ehrlisch’s test:** To 1ml of test solution, 1mlof Ehrlisch’s reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	No red colour was formed	Shows the absence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	No violet colour ring was formed	Shows the absence of tryptophan.
5.	**Test for tyrosine:** **a. Millon’s test:** To 1 ml of test solution, 2-5 drops of millon’s reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	No red colour was formed	Shows the absence of tyrosine.

	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	No green colour was formed.	Shows the absence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	No red colour was formed	Shows the absence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	No red colour was formed.	Shows the absence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	Black precipitate was formed.	Shows the presence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	Red colour was formed.	Shows the presence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	No bottle green colour was formed.	Shows the absence of methionine.

Result:

The given sample was systematically analyzed and found to be **Cysteine**.

EX NO: 6 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (ARGININE)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	No orange red colour was formed.	Shows the absence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin’s-cole’s test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	No violet colour ring was formed at the interphase.	Shows the absence of tryptophan
	ii. **Ehrlisch’s test:** To 1ml of test solution, 1mlof Ehrlisch’s reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	No red colour was formed	Shows the absence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	No violet colour ring was formed	Shows the absence of tryptophan.
5.	**Test for tyrosine:** **a. Millon’s test:** To 1 ml of test solution, 2-5 drops of millon’s reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	No red colour was formed	Shows the absence of tyrosine.

	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	No green colour was formed.	Shows the absence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	No red colour was formed	Shows the absence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	Red colour was formed.	Shows the presence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	No black precipitate was formed.	Shows the absence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	No red colour was formed.	Shows the absence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	No bottle green colour was formed.	Shows the absence of methionine.

Result:

The given sample was systematically analyzed and found to be **Arginine**.

EX NO: 7 DATE:	QUALITATIVE ANALYSIS OF AMINO ACIDS (METHIONINE)

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for amino acids :** **Ninhydrin test-** To 1ml of the test solution 2-5 drops of ninhydrin reagent was added and kept in boiling water bath.	Purple or violet colour was formed.	Shows the presence amino acids with α-amino group.
2.	**Test for protein:** **Biuret test-** To 1ml of test solution, 0.5 ml of 10% sodium hydroxide was added. Then 2-5 drops of copper sulphate was added.	No violet colour was formed.	Shows the absence of protein
3.	**Test for aromatic amino acids:** **Xanthoproteic test-** To 1ml of test solution, 1ml of concentrated HNO_3 added and cooled. To this 0.5ml of 40% sodium hydroxide was added gently.	No orange red colour was formed.	Shows the absence of aromatic acids like tryptophan,tyrosine and phenylalanine.

4.	**Test for tryptophan:** **i. Hopkin’s-cole’s test-** To 1ml of test solution,equal volume of glyoxalic acid was added. Then2-5drops of concentrated sulphuric acid was added along the sides of the test tube.	No violet colour ring was formed at the interphase.	Shows the absence of tryptophan
	ii. **Ehrlisch’s test:** To 1ml of test solution, 1mlof Ehrlisch’s reagent(10% p-dimethyl amino benzaldehyde in 10% HCL) was added and heated.	No red colour was formed	Shows the absence of indole ring containing amino acid tryptophan.
	iii. **Acrerosenhein test:** To 1ml of test solution, 1ml of formaldehyde was added and drops of 10% mercuric sulphate were added. Then 5 drops conc. H_2SO_4 was added along the sides of the test tube.	No violet colour ring was formed	Shows the absence of tryptophan.
5.	**Test for tyrosine:** **a. Millon’s test:** To 1 ml of test solution, 2-5 drops of millon’s reagent was added . The contents were shaked well and kept in a boiling water bath 10 minutes. After cooling 5 drops 1% sodium nitrate was added.	No red colour was formed	Shows the absence of tyrosine.

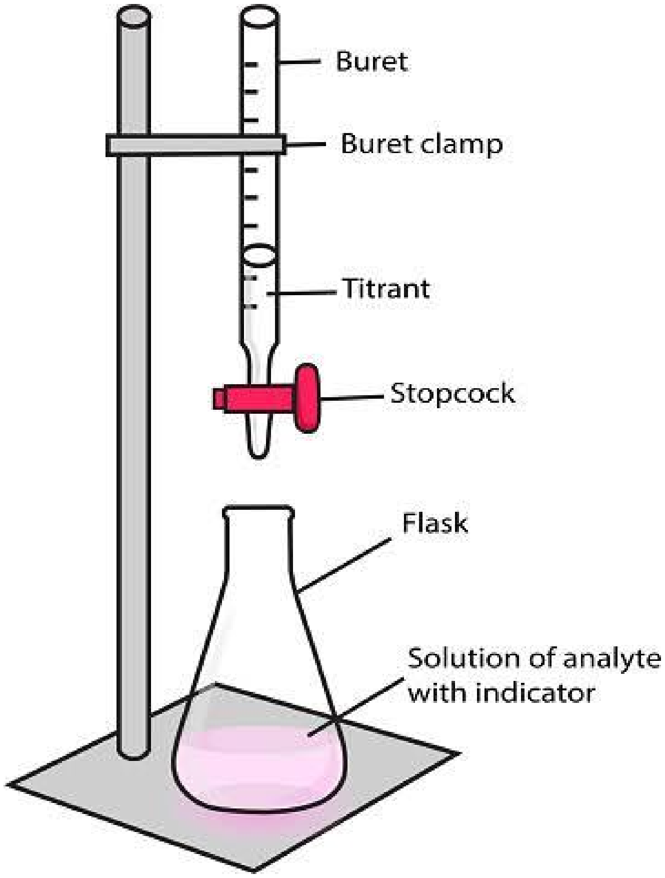
	b. Morner's test- To 1ml of the test solution, 2ml of Morner's reagent was added and kept in boiling water bath.	No green colour was formed.	Shows the absence of tyrosine.
6.	**Test for Tyrosine and Histidine:** **Pauly's test:** To 1ml of test solution, 1ml of 1% sulphanilic acid was added and cooled in ice. Then 1ml of 5% sodium nitrite was added and kept in a ice bath for 3 minutes. To this 2ml of 1% sodium carbonate was added.	No red colour was formed	Shows the absence of tyrosine or histidine.
7.	**Test for arginine:** **Sakaguchi test-** To 3ml of test solution, 1ml of 40% sodium hydroxide and 2 drops of α-naphthol was added and mixed and 4-5 drops of bromine water was added.	No red colour was formed.	Shows the absence of guanidino group containing amino acid arginine.
8.	**Test for Sulphur containing amino acids:** **Test for Cysteine and cystine:** **a) Lead acetate test :** **Preparation of Sodium Plumbate Reagent:** In a test tube ,5ml of 0.1N sodium hydroxide and 2ml of 0.1M lead acetate was added and heated directly on the flame(till the white precipitate dissolves). To 2ml of test solution was	No black precipitate was formed.	Shows the absence of cysteine and cystine.

	boiled with 0.5 ml of 40% sodium hydroxide for 2 minutes and cooled. Then 0.5ml of sodium plumbate was added.		
	b) Sodium Nitroprusside Test : To 1ml of test solution, 1ml of 1% sodium nitroprusside was added followed by the addition of 0.5 ml of 20% sodium hydroxide and kept in a boiling water bath.	No red colour was formed.	Shows the absence of cysteine and cystine.
9.	**Test for Methionine:** **Bolling's Test:** To 1 ml of test solution , 1ml of glycine , 0.5ml of 20% sodium hydroxide and 5 drops of sodium nitroprusside were added. The tube was kept in a boiling water bath for 15 minutes followed by the addition of 5 drops of 6N HCL.	Bottle green colour was formed.	Shows the presence of methionine.

Result:

The given sample was systematically analyzed and found to be **Methionine**.

TITRIMETRIC METODS



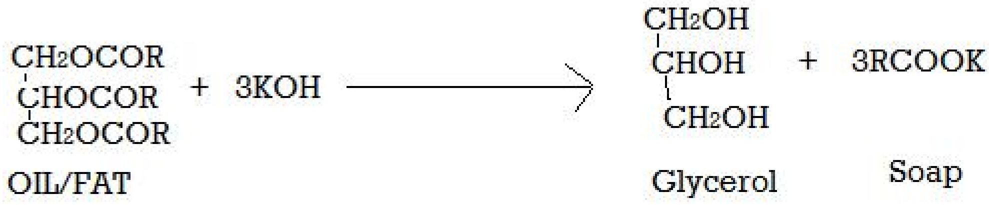
EX NO: 1 DATE:	DETERMINATION OF SAPONIFICATION VALUE OF EDIBLE OIL

AIM:

To estimate the saponification value of edible oil.

PRINCIPLE:

Saponification value is defined as the number of milligrams of potassium hydroxide(KOH) required to completely hydrolyse/saponify one gram of the fat/oil. This process is carried out with known amount of the oil or fat is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is treated against a standard acid.



Hydrolysis of fat with an alkali results in the formation of glycerol and fatty acid also called as soap.

MATERIALS AND REAGENTS:

1. Reflux & condense.r
2. Boiling water bath.
3. Burette & pipette.
4. Test components (triserine , coconut oil , corn oil , butter)
5. Fat solvents a mixture of 95% ethanol and ether[1%] 0.5 N alcoholic KOH.

Prepare 0.5 N solution of KOH of dissolving 28.05 g of KOH pellets in 20ml of water and making the volume of 1 liter with 95% of ethanol.

6. 1% phenolphthalein solution in 95% alcohol 0.5 Na Cl .

PROCEDURE:

i). Weigh accurately 1gm of fat sample in conical flask and dissolve it with 3 ml of fat solvents.
ii). Add 25 ml of 0.5 N alcoholic KOH solution. Then the reaction mixture is refluxed using a condenser on boiling water bath for 30 minutes.
iii). Cool it to the room temperature and add a few drops of phenolphthalein into the flask.

CALCULATION:

$$\text{Saponification value} = \frac{(A - B) \times F \times \text{molecular weight of KOH}}{S}$$

=

Where, S = sample weight

A. = titration volume of blank (ml)

B = titration volume of sample (ml)

F = Factor of 0.5N HCl standard solution

*** Half of molecular w eight of KOH**

iv). Titrate the content of the flask with 0.5 N HCL till the pink colour disappears.
v). Similarly, run a blank with 25 ml of 0.5N alcoholic KOH without any fat samples.

RESULT:

The saponification value of the given fat and oil is_______

EX NO: 2 DATE:	DETERMINATION OF ACID VALUE OF AN EDIBLE OIL

AIM:

To estimate the amount of acid number of an edible oil in the given sample.

PRINCIPLE:

The amount of free fatty acid can be determined volumetrically, by titrating the sample with potassium hydroxide. The acidity of fat and oil is expressed as its acid value or number which is defined as mg of potassium hydroxide required to neutralize the free fatty acid present in 1g of fat or oil. Different oil samples may contain varying amount of fatty acids in addition , the fats often become rancid during storage and this rancidity is caused by chemical or enzymatic hydrolysis of fat into free fatty acid present or acid value of fat is useful parameter which gives an indication about the age of the oil of fat.

REAGENT REQUIRED:

1. Oxalic acid (0.1 N) : 630 mg of oxalic acid in 100 ml of distilled water.
2. Potassium hydroxide (0.1N) : 5.6 g of potassium hydroxide in 1 liter of distilled water.
3. Fat solvent 95 % ethanol : ether (1%).
4. 1% phenolphthalein soluble in 95% ethanol.

Titration - I :

Determination of Acid number:

- About 5 ml of oil was measured and transferred into a clean conical flask.
- 25 ml of fat solvent was added to it shake the contents well and a few drops of alcoholic phenolphthalein solution and again mix the contents thoroughly.
- Titer the above solution with standardized potassium hydroxide. the end point was the appearance of pale pink colour which precise for 20-30 seconds.
- In another conical flask 25 ml of the fat solvent alone was taken, which serves as a blank .
- Reference between test and blank was noted.
- The amount of potassium hydroxide required to neutralise free fatty acid was calculated.

TITRATION -I

DETERMINATION OF ACID VALUE OF AN EDIBLE OIL

S NO	Contents of the conical flask (ml)	Burette reading (ml)		Volume of potassium hydroxide (ml)	Indicator
		Initial	final		
1.	Test- 5gm of oil + 25ml of alcohol				
2.	Blank - 25ml of fat solvent.				

Calculation:

Volume of standard KOH =

Normality of standard KOH =

Titer volume of sample = (T-B) ml

Acid value mg of KOH/g $= \dfrac{\text{Titer volume} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample}}$

=

RESULT:

The acid number of the oil is __________

EX NO: 3 DATE:	DETERMINATION OF IODINE NUMBER OF AN EDIBLE OIL

AIM:

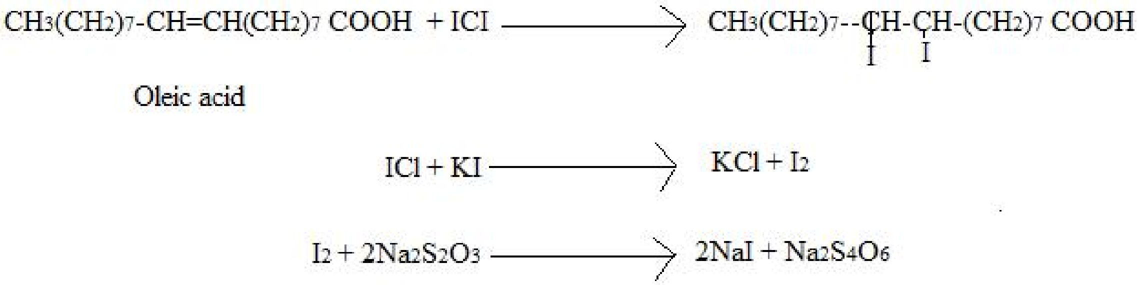
To estimate the iodine number in the given sample of oil.

PRINCIPLE:

The most important analytical determination of an oil fat is the measurement of it unsaturation. It's a useful parameter in studying oxidative rancidity of triacyl glycerol since higher the unsaturation greater is the possibility of rancidity.

Estimation of iodine number is based on treatment of known weight of fat or oil with a known volume of standard solution of iodine mono chloride and then determining the amount unused iodine mono chloride . From iodine liberated on addition of excess of potassium iodide.

The released iodine is titrated against the sodium thio sulphate solution using starch as an indicator.



$$CH_3(CH_2)_7\text{-}CH{=}CH(CH_2)_7\ COOH + ICl \longrightarrow CH_3(CH_2)_7\text{--}\underset{I}{C}H\text{-}\underset{I}{C}H\text{-}(CH_2)_7\ COOH$$

Oleic acid

$$ICl + KI \longrightarrow KCl + I_2$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

MATERIALS AND REAGENTS:

i. Stoppered bottle
ii. Burette (25 ml)
iii. Test compounds (2% solution of corn oil,olive oil, butter in chloroform)
iv. Wiji's solution.

Preparation: Dissolve 8.5 g of iodine and 7.8 ml of iodine tri chloride separately. 450 ml of acetic acid each. Mix the both solution and make the volume up to 1 liter.

v. 0.1 N sodium thio sulphate.
vi. 10% potassium iodine solution
vii. Preparation: Dissolve 100g potassium crystals in water & boil for a minute,cool and centrifuge to get the clear solution .

PROCEDURE:

Take 10 ml of fat solution in to a stoppered bottle and add 25 ml of wiji's solution. Shake thoroughly and allowed to stand in dark for 1 hour.

Calculation:

Volume of sodium thio sulphate used = (blank - test) ml

$$\text{Iodine number of fat} = \frac{\text{equivalent weight of iodine} \times \text{volume of } Na_2S_2O_3 \times 100 \times 10^{-3}}{\text{Weight of the sample (g)}}$$

=

Equivalent weight of iodine is 127
Normality of sodium thio sulphate = 0.1

Similarly prepare a blank in which for solution is replaced for chloroform.

After the reaction time of 1 hour in dark, since in the stopper and neck of the bottle with 50 ml water and 10 ml potassium iodine solution.

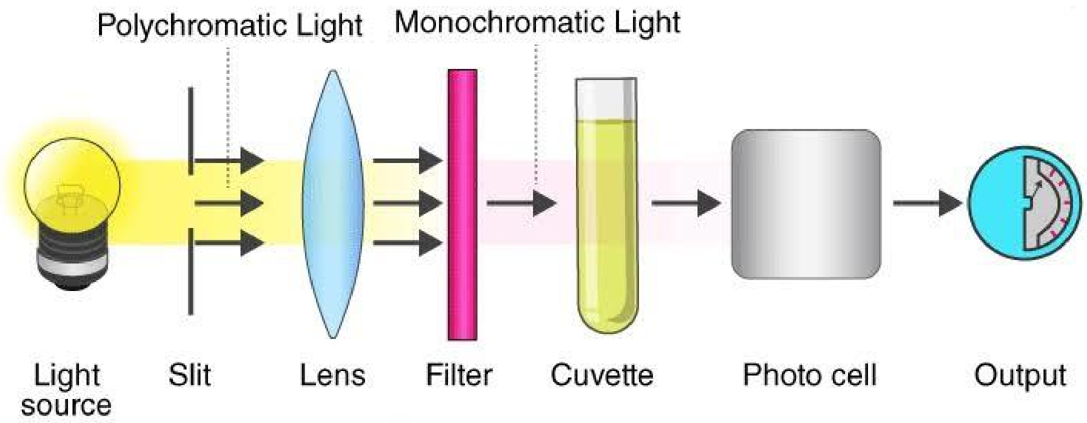
Titrate the liberated iodine with standard thiosulphate solution. Till the content of the flask become pale pink in colour.

Add few fat solution and continue titrate further with sodium thiosulphate solution till blue colour disappears.

RESULT:

The iodine number of an fat sample is ________ .

COLORIMETRIC METHODS



COLORIMETER

ESTIMATION OF PROTEIN BY BIURET METHOD

1.STOCK STANDARD PROTEIN SOLUTION:

1g of protein (egg albumin) was weighed accurately dissolved and made up to 100ml with 0.1N sodium hydroxide.

Concentration = 10mg / ml

2.WORKING STANDARD PROTEIN SOLUTION:

10 ml of the stock standard protein solution was diluted to 100ml with distilled water.

Concentration= 1mg/ml (or) 1000µg/ml

EX NO: 1 DATE:	ESTIMATION OF PROTEIN BY BIURET METHOD

AIM:

To estimate the amount of protein present in the given solution by Biuret method.

PRINCIPLE:

Cupric ions bind with the peptide bonds of protein in an alkaline medium and results in the formation of purple colour complex. The intensity of purple colour is directly proportional to concentration of protein present in the solution,which is read colorimetrically at 540nm.

REAGENTS REQUIRED:

i. STOCK STANDARD PROTEIN SOLUTION:

1g of protein (egg albumin) was weighed accurately dissolved and made up to 100ml with 0.1N sodium hydroxide.

Concentration = 10mg / ml

ii. WORKING STANDARD PROTEIN SOLUTION:

10 ml of the stock standard protein solution was diluted to 100ml with distilled water.

Concentration=1mg/ml (or) 1000 µg/ml

iii. UNKNOWN PROTEIN SOLUTION:

The given unknown solution was made up to 100 ml with distilled water.

iv. STOCK BIURET REAGENT:

45 g of sodium potassium tartarate was weighed dissolved in 100ml of 0.2 N sodium hydroxide. 15 g of copper sulphate salt was added and stirred well till it dissolves, 5g of potassium iodide was added and made up to 1litre with 0.2N sodium hydroxide.

v. DILUTE BIURET REAGENT:

200 ml of stock Biuret solution was diluted to 1 liter with 0.2N of sodium hydroxide.

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard protein solution (ml)	_	1.0	2.0	3.0	4.0	5.0	_	_
2.	Concentration of working standard protein solution (µg)	_	1000	2000	3000	4000	5000	_	_
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	2.0	4.0
4.	Volume of distilled water (ml)	5.0	4.0	3.0	2.0	1.0	_	3.0	1.0
5.	Volume of Biuret reagent (ml)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
6.	The test tubes were mixed well and incubated at room temperature (37^0C) for 10 minutes.								
7.	Optical density at 540nm								

PROCEDURE:

1ml to 5ml of working standard protein solution of concentration ranging from 1000µg-5000µg were pipetted out into series of test tubes and marked as S_1 to S_5. 2ml and 4ml of unknown solution were pipetted out into separate test tubes marked as U_1 and U_2 . The tubes were made up to 5ml with distilled water. 5ml of distilled water alone served as a blank . 3ml of biuret reagent was added to all the tubes and mixed thoroughly, then the test tubes were incubated at room temperature for 10 minutes. The purple colour was developed and measured at 540nm.

A standard graph was drawn by taking concentration of protein along x-axis and optical density along y-axis.From the standard graph, the amount of protein present in the given solution was calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to ________µg of protein.

2.0 ml of unknown protein solution contains_______µg of protein.

100 ml of protein solution contains = $\dfrac{\_\_\_\_\_\_\_\_ \times 100}{2.0}$

=

100 ml of unknown protein solution U_1 contains = _________mg of protein.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to _________µg of protein.

4.0 ml of unknown protein solution contains_______µg of protein.

100 ml of protein solution contains = $\dfrac{\_\_\_\_\_\_\_\_ \times 100}{4.0}$

=

100 ml of unknown protein solution U_2 contains = _________mg of protein.

RESULT:

The amount of protein present in the given unknown solution was found to be ___________mg/100ml.

ESTIMATION OF INORGANIC PHOSPHOROUS BY FISKE AND SUBBARROW'S METHOD.

1. STOCK STANDARD PHOSPHOROUS :

35.1 mg of potassium di hydrogen phosphate was accurately weighed, dissolved and made up to 100 ml with double distilled water.

Concentration = 80 µg/ml

2. WORKING STANDARD PHOSPHOROUS SOLUTION:

10 ml of stock was pipetted out and diluted to 100 ml with double distilled water.

Concentration = 8 µg/ml

EX NO: 2 DATE:	ESTIMATION OF INORGANIC PHOSPHOROUS BY FISKE AND SUBBARROW'S METHOD.

AIM:

To estimate the amount of inorganic phosphorous present in the given unknown solution by Fiske and Subbarrow's method.

PRINCIPLE:

The acid molybdate reagent reacts with organic phosphorous to give phosphomolybdic acid. The hexavalenic molybdenum of the acid is reduced by amino naphthol sulphonic acid to give pentavalent blue , which is read at 640nm. The intensity of the colour developed is directly proportional to the concentration of phosphorous present.

REAGENTS REQUIRED:

i. STOCK STANDARD PHOSPHOROUS :

35.1 mg of potassium di hydrogen phosphate was accurately weighed and dissolved and made up to 100 ml with double distilled water.

Concentration = 80 µg/ml

ii. WORKING STANDARD PHOSPHOROUS SOLUTION:

10 ml of stock was pipetted out and diluted to 100 ml with double distilled water.

Concentration = 8 µg/ml

iii. UNKNOWN PHOSPHOROUS SOLUTION:

The given unknown solution was made up to 100 ml with double distilled water , and then used for the experiment.

iv. AMMONIUM MOLYBDATE (2.5 %):

25 g ammonium molybdate was dissolved into 100 ml of 5 N sulphuric acid.

v. AMINO NAPTHOL SULPHONIC ACID (ANSA):

0.25 g of ANSA was weighed , to this 97.5 ml of 15% of sodium meta bi-sulphate and 2.5 ml of 20% sodium sulphate were added stirrer well and the volume was made up to 250 ml with double distilled water and stored in brown bottle.

vi. (5N) H_2SO_4.

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard phosphorus solution (ml)	_	0.5	1.0	1.5	2.0	2.5	_	_
2.	Concentration of working standard phosphorus solution (µg)	_	4	8	12	16	20	_	_
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	0.5	1.0
4.	Volume of double distilled water (ml)	9.0	8.5	8.0	7.5	7.0	6.5	8.5	8.0
5.	Volume of 2.5% ammonium molybdate (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6.	Volume of amino naphthol sulphonic acid (ml)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
7.	The test tubes were mixed well and incubated at room temperature (37^0C) for 10 minutes.								
8.	Optical density at 640nm								

PROCEDURE:

0.5 to 2.5 ml of standard phosphorous solution with the concentration ranging from 4 to 20 µg was pipetted out in to series of test tubes labelled as S_1-S_5. The given unknown solution was made up to 100 ml with double distilled water.0.5 ml and 1.0 ml of unknown solution was also taken in the different test tubes labeled as U_1 and U_2. The volume in all the test tubes were made up to 9 ml with double distilled water. 9 ml of double distilled water alone served ad blank.1 ml of ammonium molybdate and 0.4 ml of ANSA was added to all the test tubes.The intensity of the blue colour developed was read at 640nm.

A standard graph was drawn by taking the concentration of phosphorus along X axis and optical density along Y axis. From the standard graph the amount of phosphorus can be calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to _________µg of phosphorus.

0.5 ml of unknown phosphorus solution contains_______µg of phosphorus.

100 ml of phosphorus solution contains = $\frac{\_\_\_\_\_\_ \times 100}{0.5}$

=

100 ml of unknown phosphorus solution U_1 contains = _________mg of phosphorus.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to _________µg of phosphorus.

1.0 ml of unknown phosphorus solution contains________µg of phosphorus.

100 ml of phosphorus solution contains = $\frac{\_\_\_\_\_\_ \times 100}{1.0}$

=

100 ml of unknown phosphorus solution U_2 contains = _________mg of phosphorus.

RESULT:

The amount of phosphorus present in the given unknown solution was found to be _____________mg/dl.

ESTIMATION OF AMINO ACIDS BY NINHYDRIN METHOD

i. STOCK STANDARD AMINO ACID SOLUTION:

1g of alanine was weighed dissolved and made up to 100ml with acetate buffer .

Concentration = 10 mg/ml

ii. WORKING STANDARD AMINO ACID SOLUTION:

10 ml of stock standard amino acid solution was diluted to 100ml with distilled to 100ml with distilled water.

Concentration= 1mg/ml

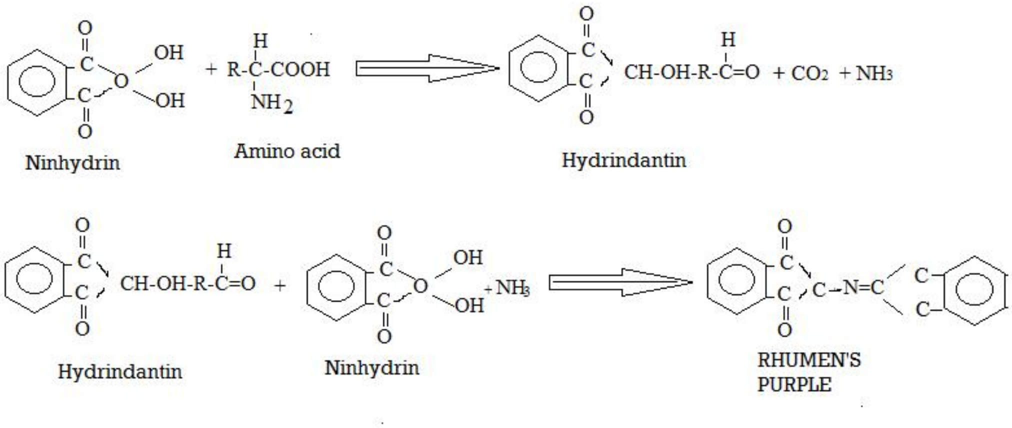
EX NO: 3 DATE:	ESTIMATION OF AMINO ACIDS BY NINHYDRIN METHOD

AIM:

To estimate the amount of amino acid present in the given unknown solution.

PRINCIPLE:

Ninhydrin is a powerful oxidizing agent and causes oxidative decarboxylation of a α-amino acid producing carbon dioxide, ammonia and aldehyde with one less carbon atom than the present amino acid, the reduced ninhydrin or hydrindantin then reacts with liberated ammonia and a molecule of ninhydrin to form a Rhumen's purple complex.



The intensity of purple colour complex developed was read at 540nm using red filter ,the colour intensity is directly proportional to the concentration of α-amino acid.

REAGENTS REQUIRED:

i. STOCK STANDARD AMINO ACID SOLUTION:

1g of alanine was weighed dissolved and made up to 100ml with acetate buffer .

Concentration = 10 mg/ml

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard amino acid solution (ml)	_	0.1	0.2	0.3	0.4	0.5	_	_
2.	Concentration of working standard amino acid solution (µg)	_	100	200	300	400	500	_	_
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	0.2	0.4
4.	Volume of distilled water (ml)	4.0	3.9	3.8	3.7	3.6	3.5	3.8	3.6
5.	Volume of Ninhydrin reagent (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6.	50% Ethanol (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
7.	All the test tubes were mixed well and kept in boiling water bath for 10 minutes and then cooled.								
8.	Optical density at 540nm								

ii. WORKING STANDARD AMINO ACID SOLUTION:

10 ml of stock standard amino acid solution was diluted to 100ml with distilled to 100ml with distilled water.

Concentration= 1mg/ml

iii. NINHYDRIN REAGENT :

2g of ninhydrin was dissolved in 25ml of methyl cello-solve to this 25 ml of 0.2 molar acetate buffer of p^H 5.5 was added. The content were mixed well and stored in brown bottle and kept away from light.

iv. ACETATE BUFFER :

Solution A: (0.2 molar acetic acid)
11.5 ml of acetic acid in 1 liter of distilled water.
Solution B: (0.2 molar sodium acetate)
27.2 g of sodium acetate in 1 liter of distilled water.

8.8 ml of solution A and 41.2 ml of solution B where mixed well and dilute to 100 ml with distilled water.

v. UNKNOWN AMINO ACID SOLUTION:

The given unknown amino acid solution was made up to 100 ml with distilled water.

vi. 50 % ETHANOL.

PROCEDURE:

0.1 to 0.5 ml of working standard amino acid solution of concentration ranging from 100-500µg where pipetted out into five different test tubes labeled as S_1 to S_5 respectively. 0.2 ml and 0.4 ml of unknown amino acid solution where pipetted out into two different test tubes labeled as U1 and U2. The volume of all the test tubes where made up to 4 ml with distilled water. 4ml of distilled water alone served as blank . Then 1 ml of ninhydrin was added to all the test tubes followed by1 ml of 50% ethanol and the tubes were kept in the boiling water bath for 10 minutes, after 10 minutes the test tube where removed and cooled. The intensity of purple colour developed was read colorimetrically at 540nm.

A standard graph was drawn by taking concentration of amino acid along x-axis and optical density along y-axis. From the standard graph the amount of amino acid present in the given unknown solution was calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to _______µg of amino acid.

0.2 ml of unknown amino acid solution contains_______µg of amino acid.

$$\text{100 ml of amino acid solution contains} = \frac{\_\_\_\_\_\_\_\_\_\_ \times 100}{0.2}$$

=

100 ml of unknown amino acid solution U_1 contains = _________mg of amino acid.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to ________µg of amino acid.

0.4 ml of unknown amino acid solution contains________µg of amino acid.

$$\text{100 ml of amino acid solution contains} = \frac{\_\_\_\_\_\_\_\_\_\_ \times 100}{0.4}$$

=

100 ml of unknown amino acid solution U_2 contains = _________mg of amino acid.

RESULT:

The amount of amino acid present in the given unknown solution was found to be__________mg/100ml.

ESTIMATION OF DNA BY DIPHENYLAMINE METHOD

i. STOCK STANDARD DNA SOLUTION:

50 mg of DNA was weighed, dissolved and made up to 50 ml of saline sodium citrate buffer.

Concentration : 1 mg/ml.

ii. WORKING STANDARD DNA SOLUTION:

5 ml of the stock standard DNA solution was diluted to 50 ml with saline sodium citrate buffer.

Concentration : 100µg/ml.

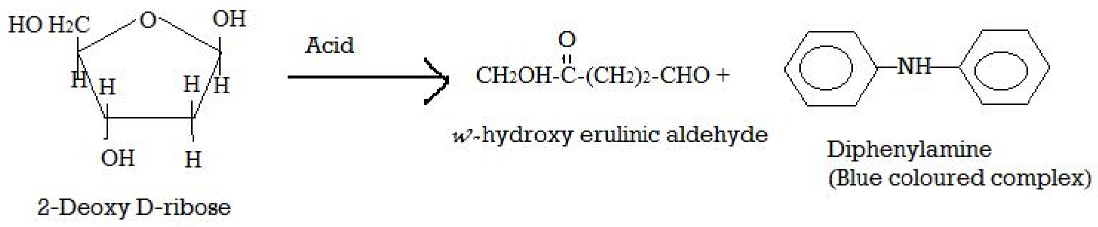
EX NO: 4 DATE:	ESTIMATION OF DNA BY DIPHENYLAMINE METHOD

AIM:

To estimate the amount of DNA present in the given unknown solution.

PRINCIPLE:

DNA is estimated using diphenyl amine reagent. Deoxyribose in DNA react in the presence of acid forms hydroxy erulinic aldehyde which reacts with diphenyl amine to give bluish green coloured complex and the colour intensity was measured at 620nm.



REAGENTS REQUIRED:

i. STOCK STANDARD DNA SOLUTION:

50 mg of DNA was weighed, dissolved and made up to 50 ml of saline sodium citrate buffer.

Concentration : 1 mg/ml.

ii. WORKING STANDARD DNA SOLUTION:

5 ml of the stock standard DNA solution was diluted to 50 ml with saline sodium citrate buffer.

Concentration : 100µg/ml.

iii. DIPHENYLAMINE REAGENT:

2.5 g of diphenylamine was dissolved in 250ml of glacial acetic acid and 3.25 ml of conc.H_2SO_4 was added and the solution was mixed out well.

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard DNA solution (ml)	–	0.5	1.0	1.5	2.0	2.5	–	–
2.	Concentration of working standard DNA solution (µg)	–	50	100	150	200	250	–	–
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	1.0	2.0
4.	Volume of distilled water (ml)	3.0	2.5	2.0	1.5	1.0	0.5	2.0	1.0
5.	Volume of Diphenylamine reagent (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
6.	All the test tubes were mixed well and kept in boiling water bath for 10 minutes.								
7.	Optical density at 620nm								

iv. PREPARATION OF UNKNOWN DNA SOLUTION:

The given unknown DNA solution was made up to 50 ml with SSC buffer.

PROCEDURE:

0.5 to 2.5 ml of working standard DNA solution of concentration ranging from 50-250 µg were pipetted out in to series of test tubes marked as S_1 to S_5. 1 ml and 2 ml of unknown solution was pipetted out in to two different test tubes marked as U_1 and U_2. The volume of all the test tubes were made up to 3ml with distilled water. 3ml of distilled water alone served as blank. To all the test tubes 5 ml diphenyl amine reagent was added. The contents were mixed well and the tubes were kept in boiling water bath for 10 minutes. After cooling the test tubes, the bluish green colour developed was read at 620nm.

A standard graph was drawn by taking concentration of DNA along x-axis and optical density along y-axis. From the standard graph the amount of DNA present in the given unknown solution was calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to ________µg of DNA .

1.0 ml of unknown DNA solution contains_________µg of DNA.

50 ml of DNA solution contains $= \dfrac{\_\_\_\_\_\_\_\_\_\_\_ \times 50}{1.0}$

$=$

50 ml of unknown DNA solution U_1 contains = _________mg of DNA.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to _________µg of DNA.

2.0 ml of unknown DNA solution contains_________µg of DNA.

50 ml of DNA solution contains $= \dfrac{\_\_\_\_\_\_\_\_\_\_\_ \times 50}{2.O}$

$=$

50 ml of unknown DNA solution U_2 contains = _________mg of DNA.

RESULT:

The amount of DNA present in the 50ml of the given unknown solution was found to be _________ mg.

ESTIMATION OF RNA BY ORCINOL METHOD

1. STOCK STANDARD RNA SOLUTION:

50mg of RNA was weighed accurately, dissolved and made up to 50 ml with saline sodium citrate (SSC) buffer.

Concentration : 1mg/ml.

2. WORKING STANDARD RNA SOLUTION:

5ml of the stock RNA solution was diluted to 50 ml with saline sodium citrate(SSC) buffer.

Concentration : 100 µg/ml.

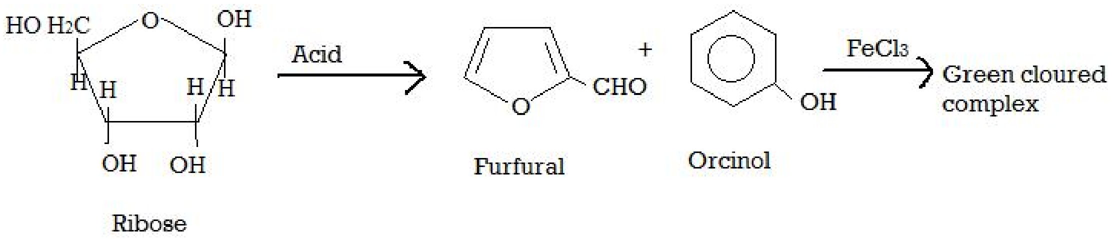
EX NO: 5 DATE:	ESTIMATION OF RNA BY ORCINOL METHOD

AIM:

To estimate the amount of RNA present in the given unknown solution.

PRINCIPLE:

RNA is estimated by using orcinol reagent. Acid hydrolysis of RNA releases ribose and this is in the presence of strong acid dehydrated to yield furfural. Orcinol reacts with furfural in the presence of ferric chloride to give green coloured complex which is measured at 680nm.



The intensity of the colour produced is directly proportional to the concentration of the substance.

REAGENTS REQUIRED:

i. STOCK STANDARD RNA SOLUTION:

50mg of RNA was weighed accurately, dissolved and made up to 50 ml with saline sodium citrate (SSC) buffer.

Concentration : 1mg/ml.

ii. WORKING STANDARD RNA SOLUTION:

5ml of the stock RNA solution was diluted to 50 ml with saline sodium citrate(SSC) buffer.

Concentration : 100 µg/ml.

iii. ORCINOL REAGENT:

300mg of orcinol was dissolved in 5 ml of ethanol. 3.5 ml of this solution was added to few ml of 0.1% of ferric chloride in the concentrated hydrochloric acid, just before use. The solution was then made up to 500 ml with concentrated HCL.

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard RNA solution (ml)	_	0.2	0.4	0.6	0.8	1.0	_	_
2.	Concentration of working standard RNA solution (µg)	_	20	40	60	80	100	_	_
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	0.4	0.8
4.	Volume of distilled water (ml)	2.0	1.8	1.6	1.4	1.2	1.0	1.6	1.2
5.	Volume of orcinol reagent (ml)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
6.	All the test tubes were mixed well and kept in boiling water bath for 10 minutes.And then cooled.								
7.	Optical density at 680nm								

iv. PREPARATION OF UNKNOWN RNA SOLUTION :

The given unknown RNA solution was made up to 50ml with distilled water.

PROCEDURE:

0.2 ml to 1.0 ml of working standard RNA solution of concentration ranging from 20-100 µg were pipetted out in to series of test tubes labelled as S_1 to S_5 respectively. 0.4 ml and 0.8 ml of unknown solution was pipetted out in to two different test tubes labelled as U_1 and U_2. The volumes of all the test tubes were made up to 2 ml with distilled water. 2 ml of distilled water alone served as blank . Then 3 ml of orcinol reagent was added to all the test tubes. The contents of the test tubes were mixed well and the tubes were kept in boiling water bath for 10 minutes. The intensity of the green colour was read at 680nm.

A standard graph was drawn by taking concentration of RNA along x-axis and optical density along y-axis. From the standard graph the amount of RNA present in the given unknown solution was calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to ________µg of RNA .

0.4 ml of unknown RNA solution contains_________µg of RNA.

50 ml of RNA solution contains = $\frac{\times 50}{0.4}$

=

50 ml of unknown RNA solution U_1 contains = _________mg of RNA.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to _________µg of RNA.

0.8 ml of unknown RNA solution contains_________µg of RNA.

50 ml of RNA solution contains = $\frac{\times 50}{0.8}$

=

50 ml of unknown RNA solution U_2 contains = _________mg of RNA.

RESULT:

The amount of RNA present in 50 ml of given unknown solution was found to be __________ mg.

ESTIMATION OF CARBOHYDRATE BY DUBOIS METHOD

1.STOCK STANDARD GLUCOSE SOLUTION:

100 mg of β- d-glucose was dissolved in 80 ml of distilled water and made up to 100 ml using distilled water.

Concentration : 1mg/ml

2.WORKING STANDARD GLUCOSE SOLUTION:

10 ml of stock glucose solution was pipetted out and made up to 100 ml using distilled water.

Concentration : 100 µg/ml

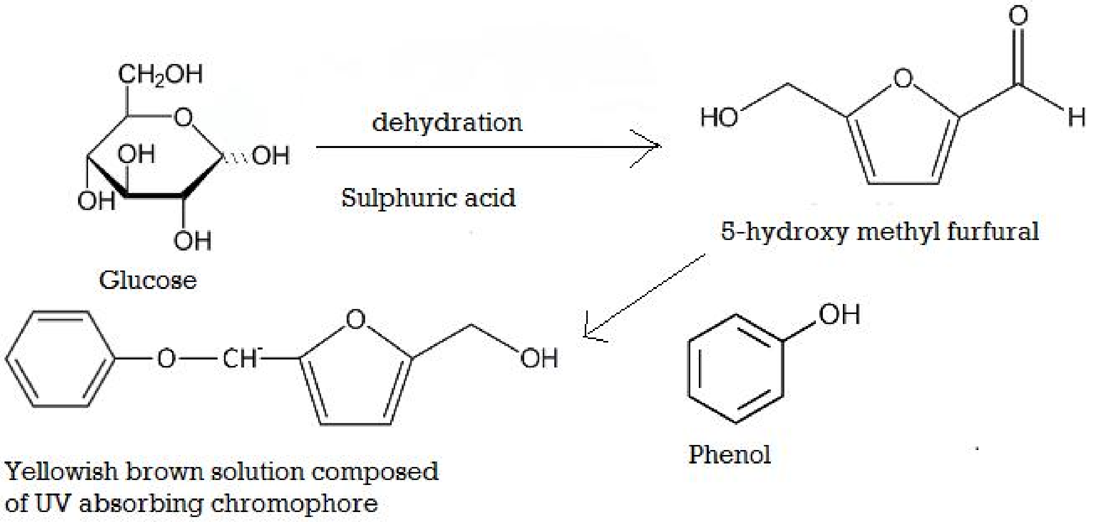
EX NO: 6 DATE:	ESTIMATION OF CARBOHYDRATE BY DUBOIS METHOD

AIM:

To estimate the amount of carbohydrate present in the given unknown solution.

PRINCIPLE:

The phenol sulphuric acid method is the easiest and reliable method among the quantitative assays for carbohydrate estimation it was originally described as a non-specific quantitative test for carbohydrates (neutral sugar content in digosaccharides, Proteoglycans, glycoprotein and glycolipids) (Dubois etal., 1956). In hot acetic medium, glucose is dehydrated to hydroxyl-methyl-furfural. This forms a yellowish brown coloured product with phenol and has absorption maximum at 490nm.


CH2OH
O
OH
OH
OH
OH
Glucose
dehydration
Sulphuric acid
O
HO
O
H
5-hydroxy methyl furfural
OH
Phenol
O
O—CH
OH
Yellowish brown solution composed of UV absorbing chromophore

REAGENTS REQUIRED:

i. STOCK STANDARD GLUCOSE SOLUTION:

100 mg of β-d-glucose was dissolved in 80 ml of distilled water and made up to 100 ml using distilled water.

Concentration : 1mg/ml

ii. WORKING STANDARD GLUCOSE SOLUTION:

10 ml of stock glucose solution was pipetted out and made up to 100 ml using distilled water.

Concentration : 100 µg/ml

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN	
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard glucose solution (ml)	_	0.2	0.4	0.6	0.8	1.0	_	_
2.	Concentration of working standard glucose solution (μg)	_	10	20	30	40	50	_	_
3.	Volume of unknown solution (ml)	–	–	–	–	–	–	0.2	0.4
4.	Volume of distilled water (ml)	1.0	0.8	0.6	0.4	0.2	_	0.8	0.6
5.	Volume of 5% phenol solution (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6.	Volume of 96% sulphuric acid (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
7.	The test tubes were mixed well and kept in boiling water bath for 10 minutes.								
8.	Optical density at 490nm								

iii. UNKNOWN GLUCOSE SOLUTION:

The given unknown solution was made up to 100 ml using distilled water and then used for further experiment.

iv. 5% PHENOL SOLUTION:

25 grams of phenol was dissolved in 400 ml of distilled water and diluted to make 500 ml using distilled water.

v. CONCENTRATED SULPHURIC ACID (H_2SO_4)

PROCEDURE:

0.2 - 1.0 ml of working standard glucose solution was pipetted out in to series of test tubes labelled as S_1-S_5. Its concentration ranging from 100-500µg. 0.2 and 0.4 ml of unknown solution was taken into the 2 different test tubes labelled as U_1 and U_2. The volume of all the test tubes were made up to 1 ml with distilled water. 1 ml of distilled water alone served as a blank. Then 1ml of 5 % phenol reagent was added to all the test tubes followed by 1ml of concentrated sulphuric acid and the tubes were mixed well and kept in boiling water bath for 10 minutes. The yellowish brown colour developed was read at 490nm .

A standard graph was drawn by taking the concentration of glucose along X axis and optical density along Y axis. From the standard graph the amount of glucose can be calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to ________µg of glucose.

0.2 ml of unknown glucose solution contains_______µg of glucose.

$$\text{100 ml of glucose solution contains} = \frac{\_\_\_\_\_\_ \times 100}{0.2}$$

=

100 ml of unknown glucose solution U_1 contains = _________mg of glucose.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to ________µg of glucose.

0.4 ml of unknown glucose solution contains_______µg of glucose.

$$\text{100 ml of glucose solution contains} = \frac{\_\_\_\_\_\_ \times 100}{0.4}$$

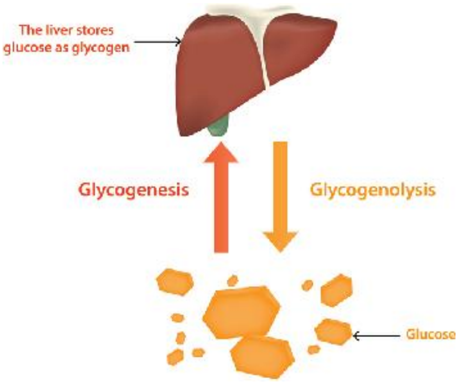
=

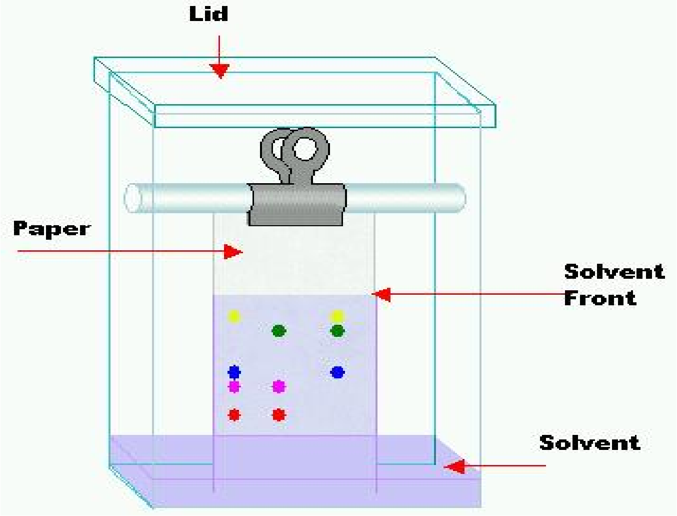
100 ml of unknown glucose solution U_2 contains = _________mg of glucose.

RESULT:

The amount of glucose present in the given unknown solution was found to be ____________ mg/dl.

GROUP EXPERIMENT





CHROMATOGRAPHY

EX NO: 1 DATE:	ISOLATION AND ESTIMATION OF GLYCOGEN FROM LIVER.

AIM:

To estimate the amount of glycogen present in the given tissue sample.

PRINCIPLE:

Carbohydrates are dehydrated by concentrated sulphuric acid to form furfural. The furfural condenses with anthrone to form a blue coloured complex. The intensity of colour is measured colorimetrically at 680nm or by using red filter.

REAGENTS REQUIRED:

1.STOCK STANDARD GLUCOSE SOLUTION:

100 mg of glucose was weighed and dissolved in 100 ml of distilled water.

Concentration: 1mg/ml.

2.WORKING STANDARD GLUCOSE SOLUTION:

10 ml of stock glucose was diluted to 100 ml with distilled water.

Concentration: 100µg/ml.

3.ANTHRONE REAGENT:

200 mg of anthrone was weighed and dissolved in 100 ml of concentrated sulphuric acid in an ice cold condition.

4.ABSOLUTE ETHANOL

5.POTASSIUM HYDROXIDE (30%)

6.PREPARATION OF SAMPLE:

1g of wet liver tissue was taken and soaked in 3ml of 30% potassium hydroxide and homogenized. The contents in tube was kept in boiling water bath for 20 minutes and cooled for saturation followed by 0.5 ml of 1N ammonium acetate it was kept overnight , next day the contents was centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded and to the precipitate 3ml of distilled water was added, then the contents was centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded and to the precipitate 5ml of boiling water was added to dissolve the precipitate.then it was cooled. From this 0.1 ml was taken for estimation.

PROTOCOL:

S.NO	CONTENTS	BLANK	STANDARD					UNKNOWN		
		B	S_1	S_2	S_3	S_4	S_5	U_1	U_2	T
1.	Volume of working standard glucose solution (ml)	–	0.1	0.2	0.3	0.4	0.5	–	–	–
2.	Concentration of working standard glucose solution (µg)	–	10	20	30	40	50	–	–	–
3.	Volume of test solution (ml)	–	–	–	–	–	–	–	–	0.1
4.	Volume of unknown solution (ml)	–	–	–	–	–	–	0.2	0.4	_
5.	Volume of distilled water (ml)	1.0	0.9	0.8	0.7	0.6	0.5	0.8	0.6	0.9
6.	Volume of anthrone reagent (ml)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
7.	The test tubes were mixed well and kept in boiling water bath for 5- 10 minutes.									
8.	Optical density at 680nm									

PROCEDURE:

0.1ml to 0.5ml of working standard glucose solution of concentration ranging from 10-50µg was pipetted out into a series of test tubes labelled as S_1-S_5. 0.1and 0.2ml of unknown solution was taken into the two different test tubes labelled as U_1 and U_2. 0.1 ml of test sample was added to the test tube labelled as T. The volume of all the test tubes were made up to 1 ml with distilled water. 1 ml of distilled water alone served as a blank.4ml of anthrone reagent was added to all the test tube and kept in boiling water bath for 5 - 10 minutes. The blue colour developed was measured colorimetrically at 680nm.

A standard graph was drawn by taking the concentration of glycogen along X axis and optical density along Y axis. From the standard graph the amount of glycogen present in the given tissue sample was calculated.

CALCULATION:

UNKNOWN SOLUTION (U_1):

_______ OD corresponds to ________µg of glucose.
0.2 ml of unknown glucose solution contains_______µg of glucose.

100 ml of glucose solution contains = $\frac{\underline{\hspace{2cm}} \times 100}{0.2}$

=

100 ml of unknown glucose solution U_1 contains = _________mg of glucose.

UNKNOWN SOLUTION (U_2):

_______ OD corresponds to ________µg of glucose.
0.4 ml of unknown glucose solution contains_______µg of glucose.

100 ml of glucose solution contains = $\frac{\underline{\hspace{2cm}} \times 100}{0.4}$

=

100 ml of unknown glucose solution U_2 contains = _________mg of glucose.

TEST SOLUTION:

_________OD corresponds to ________µg of glucose.
0.1 ml of supernatant contains_______µg of glucose.

5 ml of sample contains = $\frac{\underline{\hspace{2cm}} \times 5}{0.1}$

=

5ml of supernatant was prepared using 1g of wet liver tissue.
The amount of glucose present in 1g of wet liver tissue ________µg

=________mg

To convert the amount of glucose to glycogen the result is multiplied by the factor 0.93

The amount of glycogen in 1g of liver tissue = × 0.93

=_________mg.

RESULT:

i. The amount of glycogen present in the 1g of liver tissue was found to be_______mg.

ii. The amount of glucose present in the 1g of liver tissue was found to be ________mg.

iii. The amount of glucose present in the given unknown solution was found to be________mg.

EX.NO: 2 DATE:	SEPARATION AND IDENTIFICATION OF SUGAR AND AMINO ACIDS BY PAPER CHROMATOGRAPHY

AIM:

To separate and identify the sugar and amino acid present in the given mixture by paper chromatography.

PRINCIPLE:

Sugar and amino acid in a given mixture or sample aliquot are separated on the basis of differences in solubilities and the partition coefficient in a binary solvent. The sample with higher solubilities in stationary phase moves slowly as compared to those with higher solubilities in mobile phase. The separated sugars and amino acids are then located with corresponding spraying reagent.

REAGENTS REQUIRED :

i. WHATMANN N0.1 CHROMATOGRAPHIC FILTER PAPER

ii. LOCATING REAGENTS:

a. **For mono sugars-** 1% Potassium permanganate- 1g of potassium permanganate was weighed and mixed with 2% sodium carbonate. Charring can be continued until spots are yellow.

b. **For amino acids:** 2g of ninhydrin in ethanol. Charring can be continued until the spots are purple or blue for all amino acids except proline and hydroxy proline which gives yellow colour.

iii. SOLVENT MIXTURE:

400ml of solvent mixture which contains butanol,acetic acid and water in the ratio of 4 : 1 : 5 where prepared. The solvent was taken in a separating funnel and shaken well to separate the aqueous and organic layer. The aqueous layer is collected in a beaker and kept in a paper chromatographic chamber for saturation, where the organic is collected in a petridish and used as a running solvent for development.

iv. STANDARD SOLUTION:

1% sugar solution was prepared, the sugars used where

- ✓ Glucose (1%)
- ✓ Fructose (1%)
- ✓ Sucrose (1%)
- ✓ Lactose (1%)

1% amino acid solutions was prepared

- ✓ Arginine
- ✓ Proline
- ✓ Tyrosine

Sugar and amino acid separation must be carried out in separate chromatography paper.

PROCEDURE:

The chromatographic paper was taken and a line was drawn above 4cm from the bottom. The standard sugars and amino acid were applied at an interval of 2cm using a capillary tube the spots were allowed to dry. Unknown samples solution were also applied in the spots. The paper was dried and kept in a chromatographic chamber containing running solvent. The run was allowed till the reaches 3/4th of the paper. The paper was then removed from the chamber and allowed to dry . Then the corresponding spraying reagent was sprayed to located the samples.the distance moved by each sugar and amino acid was measured, and the R_f value was calculated.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance travelled by the solvent}}$$

The sugar and amino acid in the mixture run at different level they are measured by comparing with their standards.

CALCULATION:

FOR SUGAR:

$$R_f = \frac{\text{Distance moved by the sugar}}{\text{Distance travelled by the solvent}}$$

FOR AMINO ACIDS:

$$R_f = \frac{\text{Distance moved by the amino acid}}{\text{Distance travelled by the solvent}}$$

RESULT:

1. The sugar present in the given solution was found to be

2. The amino acids present in the given solution was found to be

REAGENTS
PREPARATION PROCEDURE

QUALITATIVE ANALYSIS

A. Reagents required for qualitative analysis of carbohydrates :

1. Molisch's reagent:

Dissolve 10 gm of α-naphthol in 100 ml of ethyl alcohol 95%

2. Iodine reagent:

Dissolve 250 mg of iodine in 100 ml of 2% potassium iodide.

3. Barfoed's reagent:

Dissolve 13.3 gm of cupric acetate in 200 ml of d.w then add 1.9 ml of glacial acetic acid.

4. Bial's reagent:

Dissolve 300 mg of orcinol in 100 ml of conc. HCL and add 0.25 ml of ferric chloride solution(10 g/ 100 ml).

5. Benedict's reagent:

Dissolve 17.3 g of $CuSO_4.5H_2O$ in 100 ml of hot water.

Dissolve separately with 173 gm of sodium citrate and 100 gm of $Na_2\,co_3$ in 100 ml of water and allow to cool.

Add citrate carbonate solution with mixing to the copper sulfate solution , dilute to 1 liter with water.

6. Fehling's reagent:

Solution A: dissolve 125 gm potassium hydroxide KOH and 173 gm sodium potassium tartarate in distilled water and dilute to 500 ml with d.w

Solution B: dissolve 34.65 gm copper sulphate in d.w dilute to 500 ml with d.w

7. Seliwanoff's reagent:

Dissolve 50 mg of resorcinol in 33 ml of conc. HCL and dilute to 100 ml with water.

8. Phenyl hydrazine (osazone)

B. Reagents required for qualitative analysis of amino acids :

1. Ninhydrin reagent:
   4g of ninhydrin in 50 ml of methyl cello solve +
   50 ml of 0.2 M acetate buffer pH s.5
   Stored in brown bottle & kept away from the light
2. Biuret reagent:
   Sodium hydroxide + CuSO4.
3. Xanthoproteic :
   Concentrated HNO_3
4. Hopkin's- cole:
   Glyoxylic acid and concentrated sulphuric acid.
5. Ehrlisch's reagent:
   $Pb(OAc_2)$ sulfanilic acid in HCl and ammonium hydroxide.
6. Acrerosenhenin reagent:
   HCHO conc sulphuric acid.
7. Millon's reagent:
   Mercury (Hg) in HNO_3
8. Sakaguchi reagent:
   10% NaOH, ɑ-naphthol,alkaline hypo bromide.
9. Pauly's reagent:
   Sulphanilic acid dissolved in concentrated sulphuric acid.
10. Sodium nitroprusside
11. Lead acetate

TITRIMETRIC METHODS

A. REAGENTS REQUIRED FOR DETERMINING SAPONIFICATION NUMBER OF AN EDIBLE OIL:

1. 0.5 N alcoholic potassium hydroxide:

14.025 g of potassium hydroxide pellets in 10 ml of water and making the volume to 500 ml with 95% of ethanol.

2. Oil sample
3. 1% phenolphthalein solution.

B. REAGENTS REQUIRED FOR DETERMINING ACID NUMBER OF AN EDIBLE OIL:

1. Potassium hydroxide (0.1N)

   2.8 g of potassium hydroxide in 500 ml of distilled water.
2. Fat solvent 95% ethanol
3. Oil sample

C. REAGENTS REQUIRED FOR DETERMINING IODINE NUMBER OF AN EDIBLE OIL:

1. Fat solution
2. Wiji's solution:

Dissolve 4.25 g of iodine and 3.9 g of iodine tri-chloride separately 225 ml of acetic acid was added. Mix the both solution & make the volume to 500 ml.

3. 0.1 N sodium thiosulphate
4. 10% potassium iodine solution.

COLORIMETRIC METHOD

Reagents preparation for 10 students.

A. REAGENTS REQUIRED FOR PROTEIN ESTIMATION BY BIURET METHOD:

1. Stock standard protein solution:

   1g of protein (egg albumin) in 100ml of 0.1 N sodium hydroxide.
2. Biuret reagent:

   4.5 g Sodium potassium tartarate, 1.5 g copper sulphate and
   0.5 g potassium iodide was made up to 100 ml with 0.2 N Na OH.

3. Dilute biuret reagent:

   100 ml of biuret made up to 500 ml with 0.2 N Na OH.

4. 0.2 N Na OH

B. REAGENTS REQUIRED FOR PHOSPHORUS ESTIMATION BY FISKE AND SUBBAROW'S METHOD :

"Double distilled water must be used"

1.Stock standard phosphorus solution:

35.1 mg of potassium di hydrogen phosphate was dissolved in 100 ml of double distilled water.

2.Ammonium molybdate (2.5%):

25 g of ammonium molybdate dissolved in 100 ml of 5N sulphuric acid.

3.Amino naphthol sulphonic acid (ANSA):

50 mg of ANSA was weighed , to this 19.5 ml of 15% sodium meta bisulphate and 0.5 ml of 20% sodium sulphate were added and stirred well. Then the volume was made up to 50 ml with double distilled water and stored in a brown bottle.

4.(5N) SULPHURIC ACID

C. REAGENTS REQUIRED FOR AMINO ACID ESTIMATION BY NINHYDRIN METHOD :

1. Stock standard amino acid solution:

1 g of alanine in 100 ml of acetate buffer.

2. Ninhydrin reagent:

4g of ninhydrin in 50 ml of methyl cello solve +
50 ml of 0.2 M acetate buffer pH s.5
Stored in brown bottle & kept away from the light

3. Acetate buffer:

Solution A: 1.5 ml of acetic acid in 100 ml of d.w
Solution B: 2.7 g of sodium acetate in the 100 ml of d.w
Solution A 4.4 ml + Solution B 20.6 ml and 50 ml of distilled water pH 5.5

D. REAGENTS REQUIRED FOR DNA ESTIMATION BY DIPHENYLAMINE METHOD:

1. Stock standard DNA solution:

50 mg of DNA in 50 ml of saline sodium citrate buffer.

2. Diphenyl amine reagent:

3.75 g of diphenyl amine dissolved + 375 ml of glacial acetic acid and 4.86 ml of con. H_2SO_4 was added and mixed well.

3. Sodium citrate buffer.

E. REAGENTS REQUIRED FOR RNA ESTIMATION BY ORCINOL METHOD:

1. Stock standard RNA solution:

50 mg of RNA dissolved in 50 ml of saline sodium citrate buffer.

2. Orcinol reagent:

150 mg of orcinol was dissolve in 2.5 ml of ethanol. 1.75 ml of this solution was added to few ml of 0.1% ferric chloride in the con. HCl just before use. The solution was then made up to 500 ml with concentrated HCL.

3. Sodium citrate buffer.

F. REAGENTS REQUIREDFOR CARBOHYDRATE ESTIMATION BY DUBOIS/ANTHRONE METHOD:

1. Stock standard glucose solution:

100 mg of β-d glucose was dissolved in 100 ml of distilled water.

2. 5% phenol solution:

5 g of phenol in 100 ml of distilled water.

3. Concentrated sulphuric acid.

GROUP EXPERIMENT

A. REAGENTS FOR ISOLATION AND ESTIMATION OF DNA:

1. 10 g of liver tissue
2. 30% potassium hydroxide
3. Ammonium acetate(1N)
4. Stock standard glucose solution:

   100 mg of glucose in 100 ml of distilled water.
5. Anthrone reagent:

   800 mg of anthrone was dissolved in 400 ml of concentrated sulphuric acid in

an ice cold condition.

B. REAGENTS REQUIRED FOR PAPER CHROMATOGRAPHY:

1. SOLVENT MIXTURE:

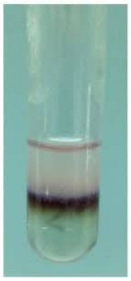
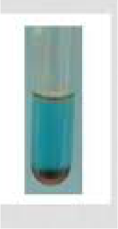
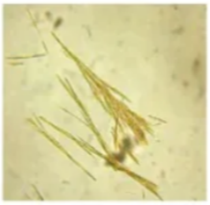
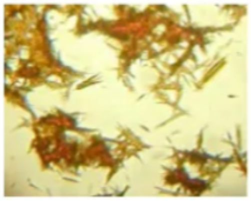
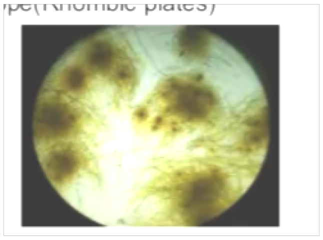
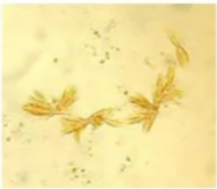
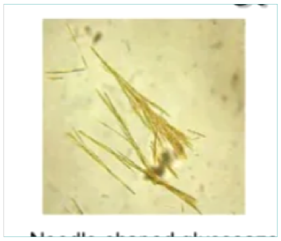
400ml of solvent mixture which contains butanol, acetic acid and water in the ratio of 4 : 1 : 5 where prepared. The solvent was taken in a separating funnel and shaken well to separate the aqueous and organic layer. The aqueous layer is collected in a beaker and

kept in a paper chromatographic chamber for saturation, where the organic is collected in a petridish and used as a running solvent for development.

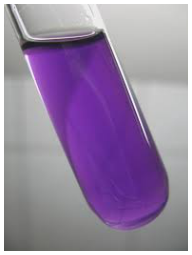
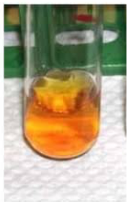
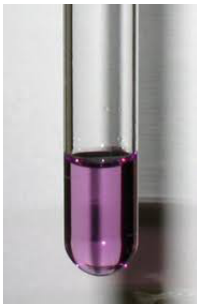
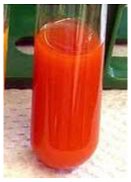
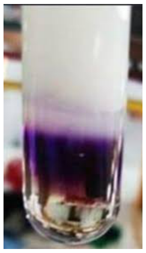
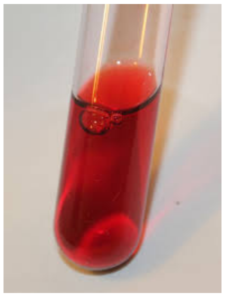
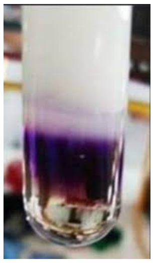
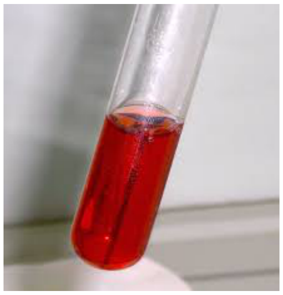
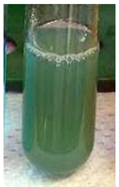
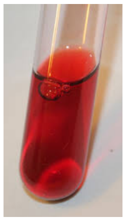
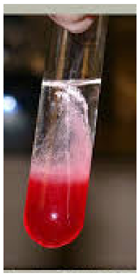
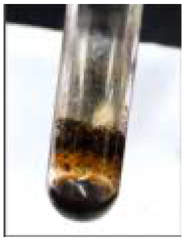
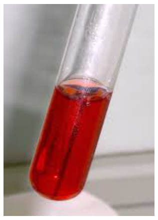
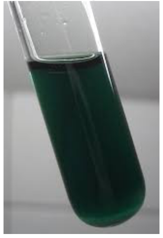
iii. 2. SPRAYING REAGENT:

c. **For mono sugars-** 1% Potassium permanganate- 1g of potassium permanganate was weighed and mixed with 2% sodium carbonate.
b. **For amino acids:** 2g of ninhydrin in ethanol.

CARBOHYDRATES-COLOUR REACTION

Molisch's test  r Medics- Lecture notes 13 Violet colour ring was formed at the junction of two liquids.	**Iodine test** Blue colour	**Barfoed's test**  Red colour precipitate
Bial's test Blue colour complex	**Benedict's test** Brick red precipitate	**Fehling's test** Red precipitate.
Seliwanoff's test Cherry red colour	**Osazone test**  Glucose,fructose,mannose, sucrose and starch- Yellow needle shape.	 Galactose- broken glass shape.
 Lactose -powderpuff shape.	 un flower shaped Maltosazc Maltose -sun flower shape.	 Xylose -long needle shape.

AMINO ACIDS- COLOUR REACTION

Ninhydrin test  a) Purple or violet colour.	 b) Yellow colour	**Biuret test**  Violet colour	**Xanthoproteic test**  Orange red colour
Hopkin's-cole's test  Violet colour ring was formed at the interphase.	**Ehrlisch's test**  Red colour	**Acrerosenhein test**  Violet colour ring	**Millon's test**  Red colour
Morner's test  Green colour	**Pauly's test**  Red colour	**Sakaguchi test**  Red colour	**Lead acetate test**  Black precipitate
Sodium Nitroprusside Test  Red colour	**Bolling's Test**  Bottle green colour		